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Reproduction and size at first maturity in a Mediterranean exploited *Callista chione* bivalve bed

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Summary: The smooth clam *Callista chione* is an Atlantic-Mediterranean species that is commercially exploited in several European countries. Several aspects of its reproduction were studied in a coastal location of the northwestern Mediterranean as a tool for sustainable fisheries management. Gonadal development was classified into 6 different stages, ranging from immature to degradation of the remaining sexual structures. Results showed that *C. chione* was able to reproduce throughout the year but a main spawning period occurred in spring. First records of hermaphrodites were found for this species. The mature spermatozoa measured $4.70 \pm 0.66 \mu\text{m}$ (without the flagellum), including a very short acrosome of $0.75 \pm 0.09 \mu\text{m}$. The mid-piece of the spermatozoa had a mitochondrial sheath composed of five mitochondria. Ripe oocytes ranged from 60 to 80 μm in length. Size at first maturity (SL_{50}) was estimated for the first time in a Mediterranean population, showing values of 30.26 mm in 2005-2006 but only 21.45 mm in 2014.

Keywords: gonads; first maturity; hermaphroditism; Veneridae; commercial mollusks.

Reproducción y talla de primera madurez en un banco de *Callista chione* explotado en el Mediterráneo

Resumen: La concha fina *Callista chione* es una especie atlántico-mediterránea comercialmente explotada en distintos países europeos. Se estudiaron varios aspectos de su reproducción en una localidad costera del noroeste Mediterráneo como herramienta para la gestión sostenible de su pesquería. El desarrollo gonadal se clasificó en 6 estadios distintos, que engloban desde individuos inmaduros a la degradación de las estructuras sexuales remanentes. Los resultados mostraron que *C. chione* se podía reproducir durante todo el año con un periodo de máxima puesta en primavera. Se observaron hermafroditas de esta especie por primera vez. Los espermatozoos maduros midieron $4.70 \pm 0.66 \mu\text{m}$ (sin el flagelo) incluyendo un corto acrosoma de $0.75 \pm 0.09 \mu\text{m}$. La pieza media de los espermatozoos estaba compuesta por 5 mitocondrias. Los ovocitos maduros medían entre 60-80 μm de longitud. La talla de primera maduración (SL_{50}) se estimó por primera vez en una población del Mediterráneo, hallando valores de 30.26 mm en 2005-2006 pero solamente de 21.45 mm en 2014.

Palabras clave: gónadas; primera maduración; hermafroditismo; Veneridae; moluscos comerciales.

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INTRODUCTION

Bivalves can provide a wide range of ecosystem functions. For example, they can improve the water quality of the ecosystems due to their filter-feeding capacity to mitigate the effects of eutrophication (Valli et al. 1994, Ostroumov 2005, Coen et al. 2007, Lindahl

and Kollberg 2009). Bivalves also promote the structures of ecosystems with their biomass, skeletons and burrowing activity (Dame 1996) and therefore contribute to the increase in habitat heterogeneity for fish and invertebrates (Gutiérrez et al. 2003, Coen et al. 2007). They can also transfer chemical elements and nutrients from the water and seston to other trophic levels and to

the sediments, linking benthic and pelagic ecosystems (Prins et al. 1998, Newell 2004). Moreover, many bivalve species play important economic roles through fisheries and aquaculture.

The smooth clam *Callista chione* (Linnaeus, 1758) is a venerid bivalve that inhabits coarse sand bottoms in the east Atlantic Ocean, from the UK down to Morocco, the Archipelago of Azores, Madeira and the Canary Islands, although it is more common in the Mediterranean Sea than elsewhere (Poppe and Goto 1993, Gofas 2013). The shell length can reach 100 mm and it is glossy and easily distinguished from any other European sea shell (Poppe and Goto 1993). The importance of *C. chione* is both ecological and economical. Baeta and Ramón (2013) reported that this bivalve was one of the main preys of the seastar species of the genus *Astropecten* in the western Mediterranean Sea, and they are also consumed by other invertebrates such as the gastropod *Hexaplex trunculus* (Morton et al. 2007) and the cephalopod *Octopus vulgaris* (Fiorito and Gherardi 1999). *C. chione* is commercially exploited and consumed in Portugal and the Mediterranean Sea (Gaspar et al. 2001, Tirado et al. 2002, Metaxatos 2004, Pubill et al. 2011, Baeta et al. 2014) where it is captured with small boats using different types of artisanal dredges. One of the main coastal shellfish areas where a major clam fishery targeted the smooth clam is the Maresme coast, located to the northeast of Barcelona (NW Mediterranean Sea). As reported by Baeta et al. (2014), the local administration commissioned a study of the smooth clam bed status in 2004 as a consequence of the decline in catches. The results prompted a temporary bed closure and an annual closing season in spring for the following four years. However, the population did not recover and the main causes of the huge reduction of the resource were overfishing and sand dredging (Baeta et al. 2014).

The reproductive system of bivalves consists of a pair of gonads, which in some species are fused in the middle (Galtsoff 1961). The gonads are made of branching tubules where gametes develop in the epithelial lining (Gosling 2003). The tubules join to form ducts leading into progressively larger ducts that terminate in a short gonoduct (Gosling 2003). Most of the bivalves are dioecious, that is, they have separate sexes. However, it is usually very difficult to distinguish the different gonads macroscopically, so the use of microscopic techniques is essential to differentiate sexes (Tirado et al. 2002). The reproductive cycle in bivalves involves gonadal development, ripeness of the gametes and spawning, but there is an enormous variation in the timing and duration of each stage both within and between species (Gosling 2003). Many exogenous (mainly temperature) and endogenous factors are involved in the reproduction processes. The role of these factors in the initiation and duration of the gametogenesis differs from their role in the timing and synchronization of spawning, a much briefer event (Seed and Suchanek 1992). The study of the reproductive cycle in bivalves is essential to understand the biology of the species and to develop an appropriate fisheries management. For example, size at first maturity is one

of the parameters commonly evaluated to assess the management of fish and shellfish stocks (Walker 1992, Williams and Babcock 2005, Camacho-Mondragón et al. 2012).

The aim of this study was to investigate the reproductive cycle of *C. chione* in the Maresme coast (NW Mediterranean Sea) and to provide the first estimation of size at first maturity in a Mediterranean population. For the purpose we used histological techniques to study the reproductive stage, sex ratios and quantitative measurements to determine oocyte diameters. We also described the spermatozoa using scanning electron microscopy. Our research reports the first case of hermaphroditism in this species.

MATERIALS AND METHODS

C. chione sampling

Specimens of *Callista chione* were collected on the Maresme coast, northeast of Barcelona (Spain), in the northwestern Mediterranean Sea (between 41°18'N, 2°20'E and 41°38'N, 2°50'E) on board an artisanal fishing boat (10 m length; 100 HP) and between 5 and 30 m depth. The gear used was a modified commercial clam dredge (mouth width, 70 cm; mouth height, 53 cm; depth, 120 cm) provided with a 12×12 mm steel mesh size smaller than the commercial size to allow juvenile individuals to be caught.

The reproductive cycle was studied using 30 individuals collected monthly from January 2005 to January 2006, except for the month of August 2005, when the collection of the samples was not possible, and the month of October 2005, when the number of individuals studied was reduced to 20.

The surface sea water temperature during the whole period of study was recorded hourly by the Wave Coastal Network of State Ports (REDCOS) (<http://www.sidmar.es/en/Services/Operational-Services/RedCos.html>) using the data from the buoy Barcelona II (41.32°N, 2.20°E).

Histological analysis

In the laboratory, the bivalves were measured with an electronic caliper and dissected, and the gonadal tissues were fixed in a buffered formaldehyde solution at 4% for 24 h. The tissues were then rinsed in tap water and transferred to 70% alcohol. The samples were dehydrated and embedded in paraffin. After processing, sections of 5-μm thickness were cut, placed in glass slides, stained using a hematoxylin-eosin staining procedure and examined under a light microscope. Sections were examined at 10×, 40× and 100× magnifications, sexed and assigned to a developmental stage according to Valli et al. (1994), i.e.: Stage 0, inactive, germinal cells are immature and the connective tissue cells are disperse in a matrix; Stage 1, early active, the gonad begins its formation with the appearance of little follicles in the connective tissue containing few germinal cells; Stage 2, late active, the gonad increases the number of follicles which contain cells in different

developmental stages; Stage 3, ripe, the gonad is fully developed with round follicles containing ripe sexual cells and occupies most of the visceral mass; Stage 4, spawning period, the follicles release the gametes and might be empty; Stage 5, degradation, the follicles might have residual gametes and the remaining sexual cells and structures are reabsorbed.

Size at first maturity

The size at first maturity (SL_{50}) is defined as the length at which 50% of the population is mature. Specimens used for the microscopic analysis of gonadal sections (from 18.8 to 95.6 mm shell length and from January 2005 to January 2006) were employed for the SL_{50} estimation. The developmental Stages 3 and 4 were considered as mature individuals and Stages 0, 1, 2 and 5 as immature for SL_{50} estimations. An additional sample of 234 specimens (ranging between 17 and 59 mm shell length) was analysed in May 2014. This month was selected because, according to the histological analysis done previously, most adult individuals were either ripe or spawning. The visceral mass was then teased apart and smears of the visceral wall with attached gonad were examined microscopically at 40x magnification. Individuals were considered to have mature gonads when (i) abundant motile sperm was observed in males and (ii) large, round oocytes with a well-defined surrounding gelatinous membrane were observed in females, according to previous studies (Metaxatos 2004).

Description of the reproductive cells and oocyte measurements

Male sexual cells were observed using a Hitachi S-3500 N scanning electron microscope located at the Institut de Ciències del Mar (CSIC), with an acceleration voltage of 15 kV, provided with back-scattered electron detector (BSE). None of the samples was coated. The size of nine spermatozoa and acrosome was measured randomly in 5 individuals during the ripening season, giving a total of 45 spermatozoa measured.

The size frequency distribution of oocyte diameters was studied monthly using the gonadal histological slides. Whenever possible, measurements involved ten females per month and reading 60 oocytes from each female using the Image-Pro Plus image analysis software package (Media Cybernetics, L.P.). To standardize the method, only oocytes with a visible nucleus were measured.

Statistical analysis

The sex ratio in the samples, expressed both as percentage of sexes and as number of males per female (M:F), was compared with parity (1:1) using the Z test for proportions. The relationship between the percentage of ripeness of the population and the water temperature was tested using the statistical software Statgraphics Plus (Manugistics, Inc., Rockville, MD, USA).

Table 1. – Characteristics of the analysed *C. chione* specimens: percentage of individuals at each gonadal stage (% ind) and minimum (Min), maximum (Max) and average (\pm SD) shell length (mm).

Gonadal stage	% ind	Min	Max	Average
0	2	56.26	57.89	57.08 \pm 0.71
1	7	31.00	66.80	49.22 \pm 9.97
2	14	33.55	76.94	59.58 \pm 8.48
3	30	41.16	91.46	66.26 \pm 9.08
4	30	40.04	95.60	65.16 \pm 10.60
5	17	18.80	82.65	52.09 \pm 13.51

Maturity relative frequencies and cumulative relative frequencies for 1-mm size intervals were computed, and the later was fitted to a logistic model from which the parameters were estimated minimizing the residual mean square between the observed data and the model.

RESULTS

Gonadal cycle

A total of 320 specimens of *C. chione* with a size range of between 18.8 and 95.6 mm were analysed for this study (Table 1). The smaller individuals measured were found in maturation Stages 1 or 5 (early active or degradation, respectively). The histological study showed that *C. chione* was able to reproduce throughout the year although a major spawning period was found in spring, specifically in May (Fig. 1A, B and C). Females seemed to have a slightly more advanced cycle than males and some spawning occurred in February, while males began in March, although there was a common major spawn in May (Fig. 1A, B). The monthly relative frequency of the gonadal stages of *C. chione* showed the simultaneous occurrence of different stages of development year-round, regardless of the sex (Fig. 1C). The gonadal maturation of the smooth clam population began in December and the gonads remained mainly ripe during February and March. In April, about 50% of the population, mainly females, was spawning. The percentage of spawning individuals reached 90% in May, which was the most important spawning period of *C. chione* for both sexes at the site. From June to November at least four different gonadal stages were found per month, including very early stages of gonadal development to spawning and degradation of the gonads after spawning. The interstitial connective tissue had different stages of development according to the ripeness of the gonad. When gonads were not ripe and Stages 0 and 1 were found, the connective tissue was well developed, but it progressively disappeared as the gonadal follicles began to form (Fig. 2). After spawning and degrading of the remaining sexual structures, the interstitial connective tissue started to develop again.

Monthly average water temperature (Fig. 1C) showed minimum and maximum values of 12.4 \pm 0.01°C (March 2005) and 26.0 \pm 0.24°C (August 2005). There was a significant relationship ($p=0.0004$) between the percentage of ripe individuals in the population and the water temperature (Fig. 3), that is, the lower the

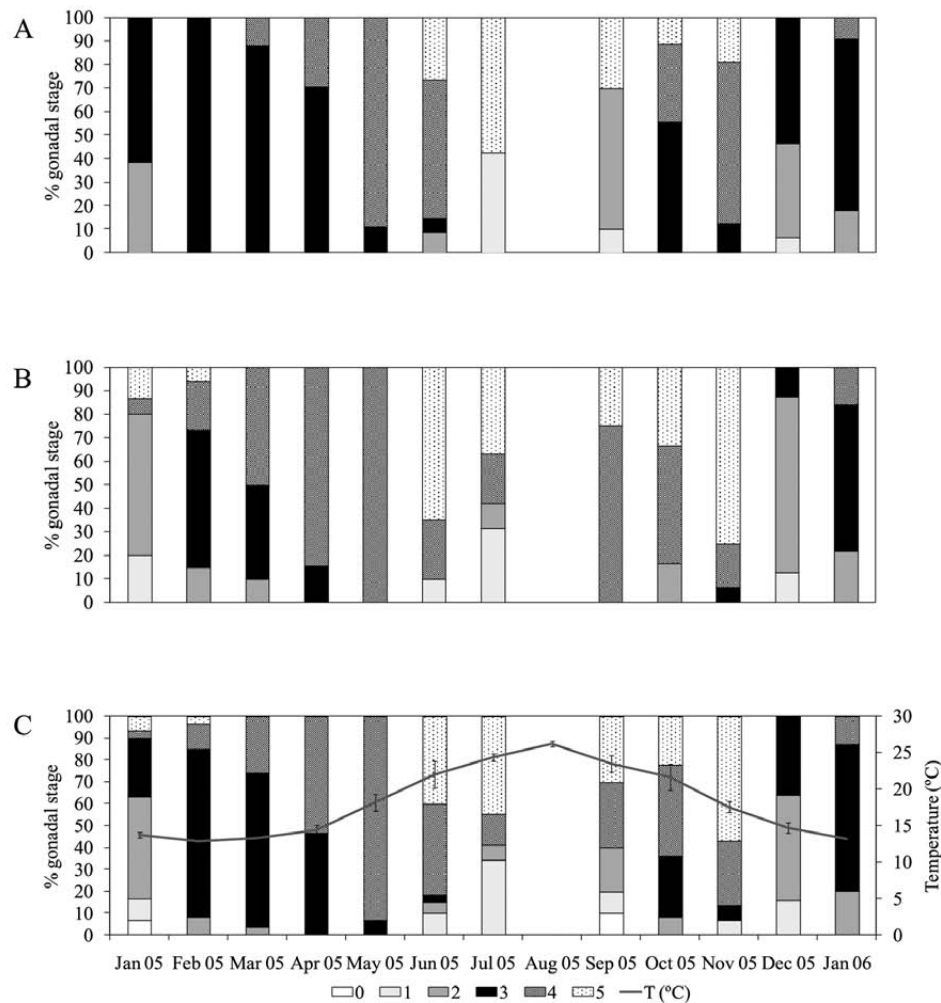


Fig. 1. – Monthly average temperature and relative frequency of the different gonad development stages throughout the period of study for males (A), females (B), and the whole population (C). Stage 0, inactive; Stage 1, early active; Stage 2, late active; Stage 3, ripe; Stage 4, spawning period; Stage 5, degradation.

temperature, the riper the population. The relationship found can be expressed as

$$\% \text{ ripe } C. \text{ chione} = 308.72 - 98.53 \ln \text{ temperature};$$

$$R^2 = 72.94$$

Sex ratio

It was not possible to determine sex for only 7 individuals (sizes ranging from 55.4 to 77.2 mm length) collected in the months of July and October 2005, and January 2006. Sex ratio was 1:1 in 2005-2006 (157 males vs 156 females; $Z=0.06$) and 2014 (109 males vs 95 females; $Z=0.98$). In 2014, it was not possible to determine sex in 30 individuals from the 237 analysed. The proportions of sexes were not significantly different ($Z=0.73$) between 2005-2006 and 2014. The critical value for $\alpha=0.05$ is $Z(0.05)=1.96$; all obtained statistics were smaller.

We report for the first time the existence of hermaphrodites in *C. chione*. Within the histological samples we found five cases: two individuals from March 2005, two from September 2005, and one from January 2006 (Fig. 4). From the smear samples in 2014

we identified one hermaphrodite. The frequency of occurrence was 1.4% and 0.4% in 2005-2006 and 2014, respectively.

Size at first maturity

Size at first maturity was analysed microscopically from two data sets: histological slides of the gonad in 2005-2006 and smears of the gonad in 2014. The logistic model was fitted to both data sets minimizing the sum of squares of the differences between the frequencies observed and estimated by the model. In Table 2 the general statistics and the overall results are presented. Results for 2005-2006 showed that SL_{50} was 30.26 mm. Nevertheless, mature females with a 20.6 mm length and mature males as small as 23.8 mm were observed. For 2014, SL_{50} was 21.45 mm. The residual mean square shows a better fitting of 2005-2006 data than 2014 data. Furthermore, comparing both fittings it can be seen that the estimated logistic growth parameter (r) is higher for 2014 data and lower for 2005-2006, meaning that the logistic curve is sharper for 2014; this can also be seen considering the difference between the quartiles $SL_{75}-SL_{25}$, 13.8 mm for 2005-2006 and 9.5

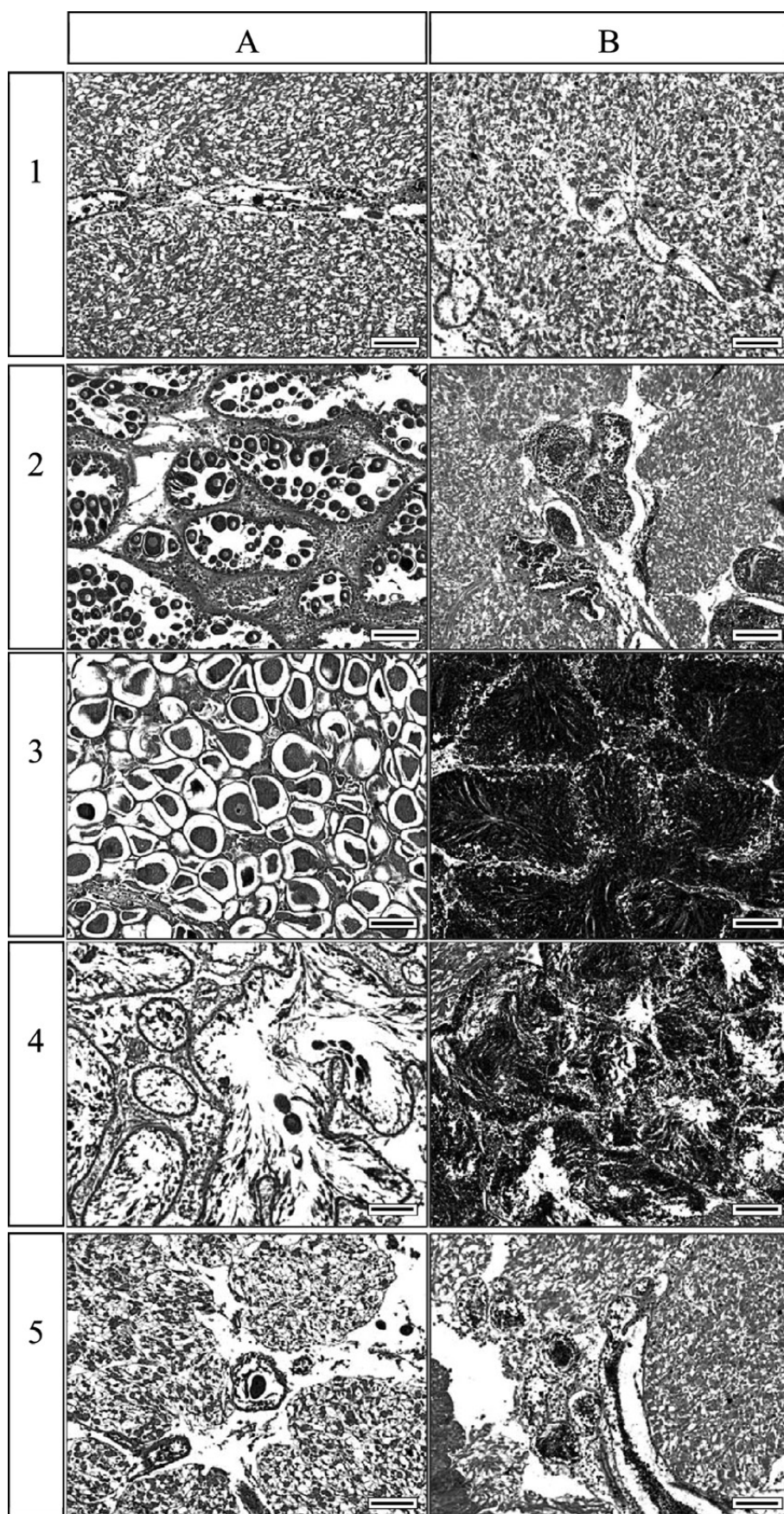


Fig. 2. – Histological sections of the gonads showing different gonad stages for both sexes. Columns: A, female sections; B, male sections. Rows: 1, Stage 1; 2, Stage 2; 3, Stage 3; 4, Stage 4; 5, Stage 5. Scale bar measures 100 μ m.

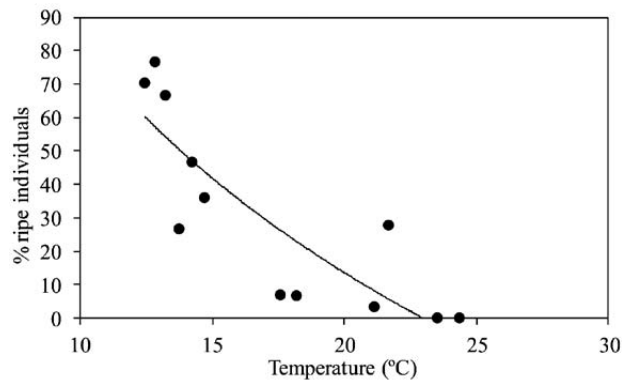


Fig. 3. – Relationship between the percentage (%) of ripe individuals in the population and the water temperature (°C).

Table 2. – Basic sample statistics and fitting values of the logistic model to estimate the size at first maturity (SL_{50}) for both sampling periods (years 2005-2006 and 2014).

	2005-06	2014
Number of classes	71	33
Number of individuals measured	320	234
Lower observed length (mm)	17	17
Lower length with mature individuals (mm)	18	19
Higher length with immature individuals (mm)	58	28
Higher observed length (mm)	95	59
SL_{25} (mm)	23.37	16.70
SL_{50} (mm)	30.26	21.45
SL_{75} (mm)	37.15	26.21
r (growth parameter of the logistic model)	0.1593	0.2309
Residual mean square	0.018	0.025

mm for 2014. The size structure of the sampled specimens changed between 2005-2006 and 2014, with the biggest clams missing in the second period of the study (sizes between 59 and 95 mm length) because of their absence in the population.

Description of the reproductive cell and oocyte measurements

Mature spermatozoa measured $4.70 \pm 0.66 \mu\text{m}$ (Fig. 5) (without the flagellum), including a very short acro-

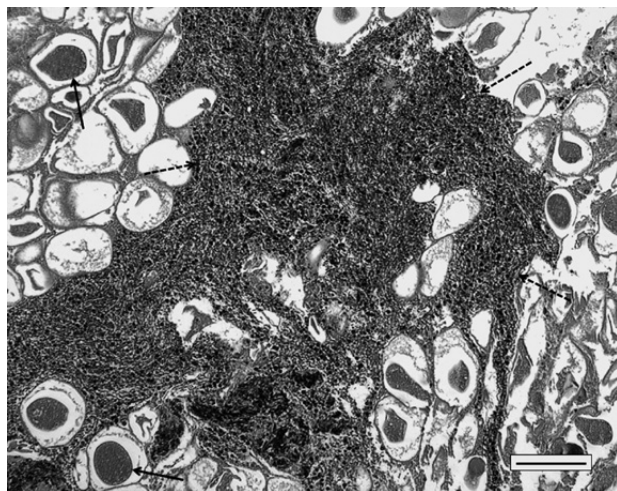


Fig. 4. – Histological section of the gonads of a hermaphrodite individual. Arrows point to mature oocytes; broken arrows point to the mass of sperm. Scale bar is 100 μm .

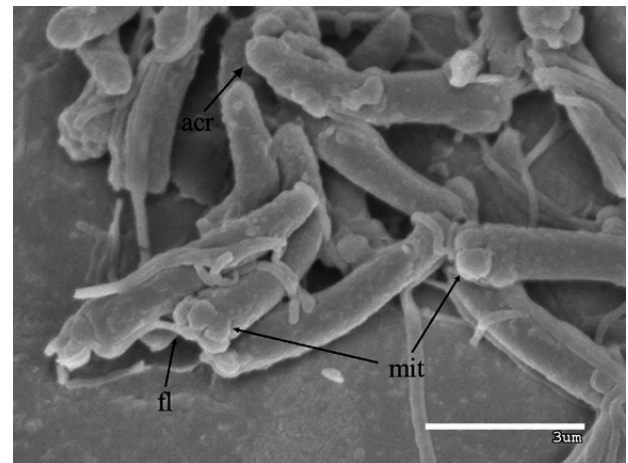


Fig. 5. – Scanning electron microscope image of a male gonad section. Acr, acrosome; fl, flagellum; mit, mitochondria. Scale bars shown in image.

some of $0.75 \pm 0.09 \mu\text{m}$ length. The mid-piece of the spermatozoa has a mitochondrial sheath composed of five mitochondria; the tail emerged from the middle of the sheath.

Most of the oocytes observed in the histological samples were round and, when fully ripe, they were much hydrated (average diameter $58.48 \pm 17.42 \mu\text{m}$). Nevertheless, in Stages 2 and 4, oocytes were occasionally linked to the gonadal follicle through a peduncle (pedunculated oocytes) and their sizes ranged between 35 and 171.34 μm . Ripe oocytes from *C. chione* had a nucleus, which occupied much of the reproductive cell and was stained in pink with the stains used in our study. Inside the nucleus there was a nucleolus, which might seem bi-lobulated in some cases but was just the observation of the two components of the nucleolus: pars granulosa, with granules of rRNA and proteins; and pars fibrosa, with filaments of rRNA (Fig. 6).

The length frequency distributions of oocyte diameters according to sampling months are shown in Figure 7. For most of the months, the frequency distributions of oocyte diameters were polymodal, indicating female

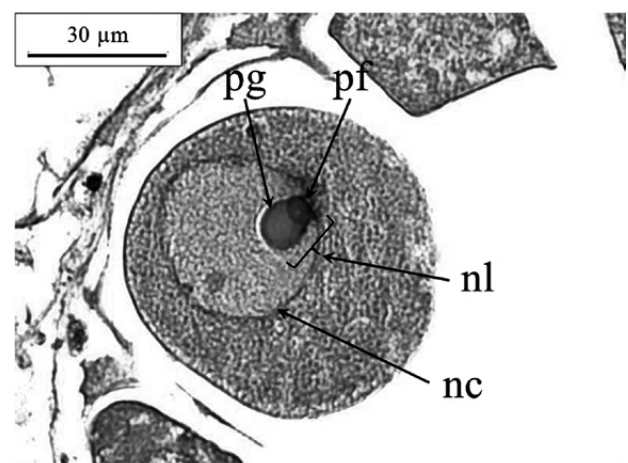


Fig. 6. – Optical micrographs of a ripe oocyte with a nucleus (nc) and a nucleolus (nl) and its two parts: pars granulosa (pg) and pars fibrosa (pf). Scale bars are 30 μm .

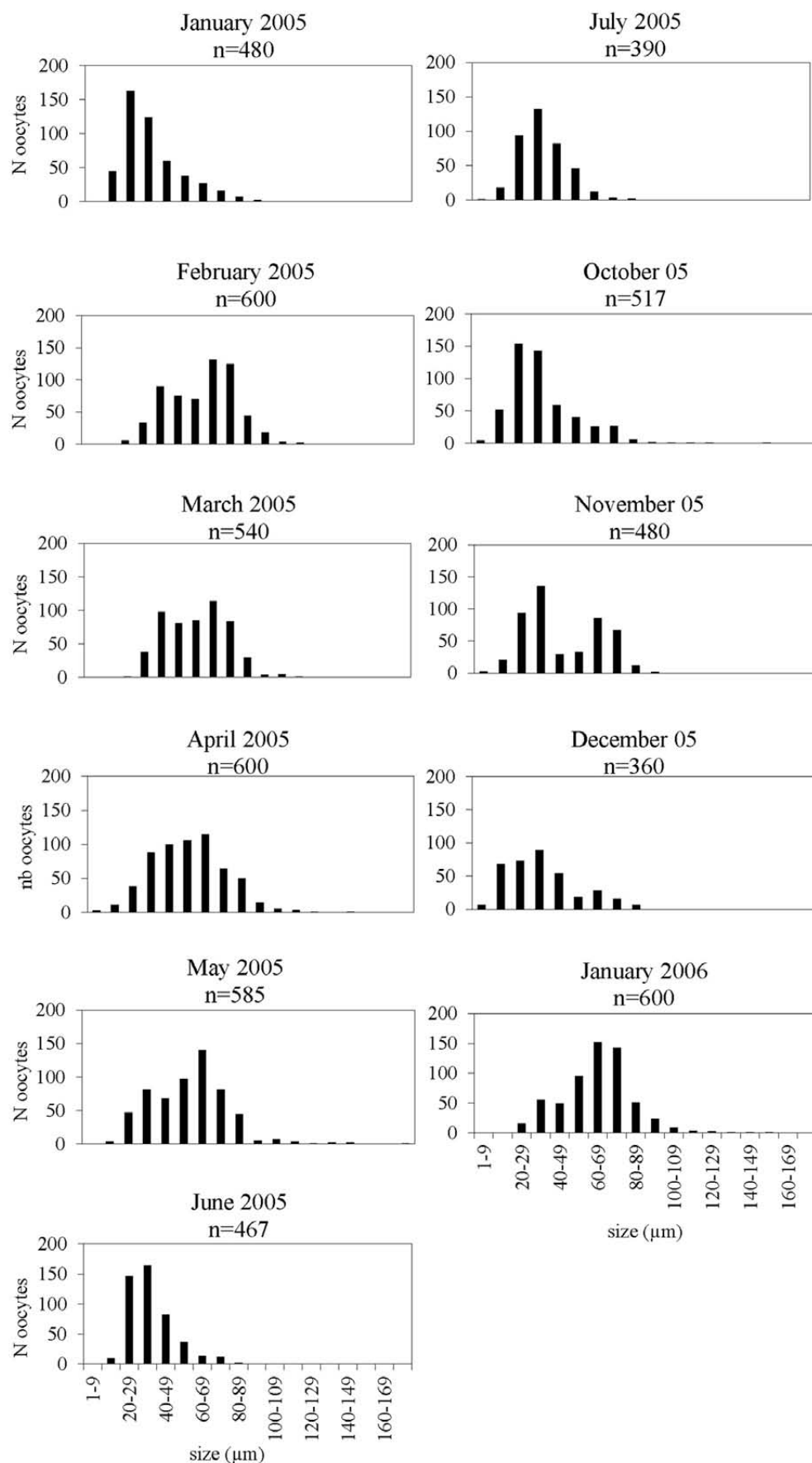


Fig. 7. – Frequency distribution of the oocyte lengths during the whole study period. N indicates the number of oocytes measured.

Table 3. – Monthly minimum (Min), maximum (Max), and average (\pm SD) oocyte sizes per month throughout the study period. N is the number of oocytes counted out of the number of individuals indicated in the ind column.

	Min	Max	Average	N	ind
January 05	15.08	98.68	36.62 \pm 16.03	480	8
February 05	15.55	119.83	59.14 \pm 19.21	600	10
March 05	19.41	115.79	55.37 \pm 17.80	540	9
April 05	7.87	140.43	56.00 \pm 20.11	600	10
May 05	18.67	171.34	58.21 \pm 20.80	585	10
June 05	15.08	88.97	37.02 \pm 12.54	467	8
July 05	8.00	82.65	37.50 \pm 12.31	380	8
October 05	8.94	156.21	37.76 \pm 18.56	517	9
November 05	7.77	97.44	46.47 \pm 20.08	480	8
December 05	8.43	87.81	36.03 \pm 18.08	360	6
January 06	23.29	152.78	64.74 \pm 18.35	600	10

gametes in different developmental stages. The highest frequency of oocytes (measuring $>60\text{ }\mu\text{m}$) was seen in January 2006 (64.33%).

In January 2005 most of the oocytes were small, coinciding with most of the females developing the gonad. In February and March 2005, the oocytes had grown and a few of them were pedunculated. In April and May 2005 there were small oocytes, observed in spawned *C. chione* (stage 4), and big oocytes in ripe smooth clams. In June and July 2005 the oocytes were small because the gonads were regenerating or degrading. The same stages were found in September 2005, when measurable oocytes with a visible nucleus were too few, so measurements could not be taken. October was similar to the summer months. In November, oocytes 60–79 μm large were found in ripe gonads, and the largest oocytes were pedunculated; the smaller oocytes were found in females that were spawning or degrading the gonads. In December 2005 there were no pedunculated oocytes. Finally, in January 2006 most of the oocytes were large and ripe with some pedunculated oocytes, which were the largest oocytes found.

The monthly maximum, minimum, and average oocyte sizes per month are reported in Table 3. The length of the smaller oocytes found throughout the study ranged from 7.77 μm in November 2005 to 23.29 μm in January 2006. The maximum oocyte lengths ranged from 82.65 μm in July 2005 to 171.34 μm in May 2005. Monthly mean oocyte diameter varied from 36.03 \pm 18.08 μm (December 2005) to 64.74 \pm 18.35 μm (January 2006). The highest average lengths were counted in months in which *C. chione* were ripe or spawning and the shorter average oocytes were found in the months in which the gonads were developing and/or degrading.

DISCUSSION

The bivalve *C. chione* is able to reproduce the whole year round with a spawning peak in spring. The gonadal development of this species had been previously studied through histological analysis on two occasions in the Mediterranean Sea (Valli et al. 1994, Tirado et al. 2002). In those studies, *C. chione* was also found to be capable of reproducing the whole year round. Tirado et al. (2002) observed three spawning peaks in southern Spain, finding the main emission of gonads

in spring, as observed in our study. Valli et al. (1994) observed that the spawning period occurred in spring and summer in the Gulf of Trieste, with individuals increasingly spawning until a major peak in August. The gametogenic cycle of *C. chione* described in Portugal reported three spawning peaks and the release of the gametes lasted from January to October. Water temperature and nutrient supply are known to directly affect the physiological status of bivalves, regulating gametogenic cycles and spawning, among other physiological processes (Wilson and Hodgkin 1967, Hilbish and Zimmerman 1988, Gosling 2003, Morgan et al. 2013). In agreement with these studies, we found a very strong relationship between the amount of ripe individuals and the water temperature. Therefore, we hypothesize that the differences and similarities found between the gonadal cycle reported in the present study and the previous ones are most likely caused by temperature and food supply inter-site variations, though data on food supply are needed to support this hypothesis.

The sex ratio of *C. chione* was 1:1, as previously described in other populations (Tirado et al. 2002, Metaxatos 2004, Moura et al. 2008). Our study reports the first cases of hermaphroditism for *C. chione*. In the Veneridae, hermaphrodite individuals have been found in two different species, *Ruditapes philippinarum* (Delgado and Pérez-Camacho 2007, Lee et al. 2013), with frequency values similar to our findings (Drummond et al. 2006), and *Megapitaria squalida* (Romo Piñera et al. 2009). The high hermaphrodite frequency (between 21.8% and 23.5%) for *M. squalida* was related to the low population density resulting from overfishing (Romo Piñera et al. 2009). The smooth clam population studied here was also affected by overfishing, but the frequency of hermaphrodites was much lower than what reported by Romo Piñera et al. (2009) for *M. squalida*. Therefore, we do not think that overfishing would cause hermaphroditism in *C. chione*, as hermaphroditic individuals have occasionally been found in bivalve species strictly considered gonochoric.

There are some discrepancies in the size of *C. chione* oocytes: whereas Metaxatos (2004) reported oocyte sizes ranging from 54.5 to 130 μm and Pérez-Larruscain et al. (2011) described a mean oocyte diameter of 89.5 μm under laboratory conditions, Moura et al. (2008) reported a ripe oocyte size of only 36 μm diameter. Our results agree with the studies done in the Mediterranean Sea, i.e. Metaxatos (2004) and Pérez-Larruscain et al. (2011), finding bigger sizes which are in the size range of other venerid species (Mouëza et al. 1999, Chung 2007, Popović et al. 2013). The differences observed in the size of oocytes from Atlantic and Mediterranean smooth clam populations were related by Moura et al. (2008) to the tissue sample state (preserved or fixed) and also to genetic differences. The size of the sperm cells and acrosome agree with those described by Nicotra and Zappata (1991) in *C. chione* and are in the size range of other Veneridae species (Gwo et al. 2002).

Most of the *C. chione* dissected in the present study had developed sexual structures, in contrast to previ-

ous studies, including the smallest bivalves analysed in both periods (18.8 mm and 20.6 mm length in 2005-2006 and 2014, respectively). Moura et al. (2008) reported that individuals might start spawning at 30 mm length, and the size at first maturity was estimated at a shell length of 50.81 mm on the southwestern coast of Portugal. In a later work, the same authors reported that *C. chione* needed more than four years to reach sexual maturation (Moura et al. 2009). In contrast, Metaxatos (2004) studied smears of *C. chione* gonads and reported that 25% of one-year-old smooth clams could be sexed, but gonadal maturation for the whole population began at the age of two years old. The author also reported that gonads were discerned in *C. chione* individuals larger than 12 mm. Size at first maturity found in the Mediterranean for *C. chione* in this study is much smaller than that reported by Moura et al. (2008) in the NW Atlantic. There is no agreement on the best method for estimating the size at first maturity in bivalves, as there are differences in the method of observation of the gonads and/or the period of the reproductive cycle sampled. Some authors analyse histological sections of samples collected in the time period when adult specimens are ripe (Moura et al. 2008, Delgado et al. 2013), which requires previous knowledge of the reproductive cycle of the species. Other authors estimated the SL_{50} using samples collected monthly throughout one year, using two different methods: microscopic analysis of histological sections of the gonads (Camacho-Mondragón et al. 2012) or microscopic examination of smears of the sexual products (Derbali et al. 2009). Despite these differences, all methods are considered appropriate and commonly used for the SL_{50} estimation.

It is a common fisheries management practice to impose a minimum legal catch size greater than the size at first maturity, in order to allow the individuals to participate in spawning for some time before being fished (Williams and Babcock 2005). In this study we report a decrease in the SL_{50} for a *C. chione* population, which is to our knowledge the first mention in an overexploited bivalve population. We cannot assure that the nearly 9 mm SL_{50} decrease observed between 2005-2006 and 2014 was not affected by the different methodology used here to determine the value in each period, but the results are beyond the particular value and strongly suggest a downward trend. From a management point of view, this information is important to promote the periodical revision of this parameter as changes in the life history traits of the species occur over time. Changes in size and age at first maturity in many fish stocks often occurred after the stock abundances declined sharply. For example, after stock collapses in the early 1990s, both size and age at first maturity declined sharply for the cod (*Gadus morhua*) stocks in the northwest Atlantic (Saborido-Rey and Junquera 1998, Hutchings 2005). In mollusks, including gastropods and bivalves, differences in size at first maturity have been reported between populations of the same species (Hooker and Creese 1995, Cruz et al. 2000). In the gastropod *Halotis iris*, such differences might be related to differences in growth rates between populations (Hooker and Creese 1995). Furthermore,

McShane and Naylor (1995) found differences in size at maturity and growth rate of the gastropod *H. iris* at small spatial scales between headlands and bays separated by as little as 200 m. Cruz et al. (2000) reported that the differences in age and size at first maturity of the bivalve *Argopecten ventricosus* from two populations might be a consequence of both internal triggering mechanisms and environmental conditions. Changes in the biology of the species may also represent a plastic response to changes in the environment, such as compensatory density-dependence in growth and maturation, or a direct response of maturation to increased body condition or to temperature. In this study no environmental information was available (chlorophyll *a*, seston concentration), apart from surface sea water temperature, to investigate the possible causes for the reduction in SL_{50} , but our results reinforce the need to revise this tool periodically to design management strategies.

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